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In-vivo assessment of *in-vitro* killing patterns of *Pseudomonas aeruginosa*

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Time-kill curves of *Pseudomonas aeruginosa* exposed to gentamicin or ticarcillin *in vitro* were correlated with time-kill curves obtained with various dosage schedules of the same study drugs in granulocytopenic mice. An instantaneous, fast and drug-dependent killing pattern was found *in vitro* with gentamicin. This pattern corresponded to bacterial killing *in vivo* which was clearly dependent on peak drug levels. In contrast, slow bacterial killing with little relationship to concentration was found *in vitro* with ticarcillin and proved to correlate with an antibacterial effect *in vivo* seen at trough levels. We conclude that *in-vitro* time-kill curves of antimicrobial agents may be predictive for optimizing dosage regimens *in vivo*.

Introduction

The *in-vitro* activity of an antibiotic can be well established in time-kill curves as obtained by exposure of the test bacteria to various concentrations of drug. In similar experiments, the regrowth pattern of bacteria can be studied *in vitro* after short exposure of the organisms to the study drug. Using such technique, Bundtzen *et al.*, (1981) and Gerber & Craig (1981) have shown that exposure of various Gram-negative bacteria to inhibitors of protein- or nucleic acid-synthesis is followed by a persistent post-antibiotic effect (PAE); i.e. a persistent suppression of bacterial growth upon removal of the drug. In contrast, virtually no PAE was found after exposure of Gram-negative rods to β-lactam drugs.

Little is known about how killing and regrowth patterns observed *in vitro* relate to antimicrobial activity of antibiotics *in vivo*. In the present study, therefore, experiments were devised to establish time-kill curves of the same pairs of drug and organism not only *in vitro* but also *in vivo*. A hypothesis was proposed that *in-vivo* efficacy of an antimicrobial agent is related to peak plasma levels whenever a fast bactericidal drug effect and a PAE are found *in vitro*, whereas slow bacterial killing and absence of a PAE *in vitro* indicate that *in-vivo* antimicrobial activity depends not on peak- but mainly on trough drug levels.

Materials and methods

Pseudomonas aeruginosa ATCC 27853 was the main study organism. Two clinical isolates of *Ps. aeruginosa* were included in confirmatory experiments. Ticarcillin

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(Beecham) and gentamicin (Shering, U.S.A.) were the drugs used. *In-vitro* time-kill curves were constructed after exposure of the organisms to various concentrations of drug in Mueller–Hinton broth supplemented with Ca^{++} and Mg^{++} according to Stratton & Reller (1977). The number of colony-forming units (cfu) was determined by plating serial dilutions of appropriate culture samples onto tryptic soy agar plates. Methods for *in-vivo* studies have been described by Gerber, Craig & Brugger (1983). Agranulocytic (cyclophosphamide treated) mice were used. On the day of the experiment, mice were injected with 10^7 cfu of the organisms in the thigh muscle. Two hours later treatment was started with sc injections of the study drug. In some experiments mice were randomized and treated with either a 1-h or a 3-h regimen such that identical total amounts of injected drug resulted in both groups after 3, 6, 9 and 12 h of treatment. Blood was drawn from the retro-orbital sinus of all mice, and plasma drug levels were determined using a biological method (*Bacillus subtilis* as indicator organism). Antimicrobial activity was assessed by viable counts in homogenized thighs of killed animals, and time-kill curves were constructed.

Results

Killing patterns of Ps. aeruginosa in vitro

Time-kill curves of *Ps. aeruginosa* ATCC 27853 obtained *in vitro* with various concentrations of gentamicin and ticarcillin are shown in Figure 1. A substantial difference between the two drugs was found regarding the time course of the bactericidal effect. Killing by gentamicin was fast, very much concentration-dependent, but followed by bacterial break-through growth. On the other hand, ticarcillin needed

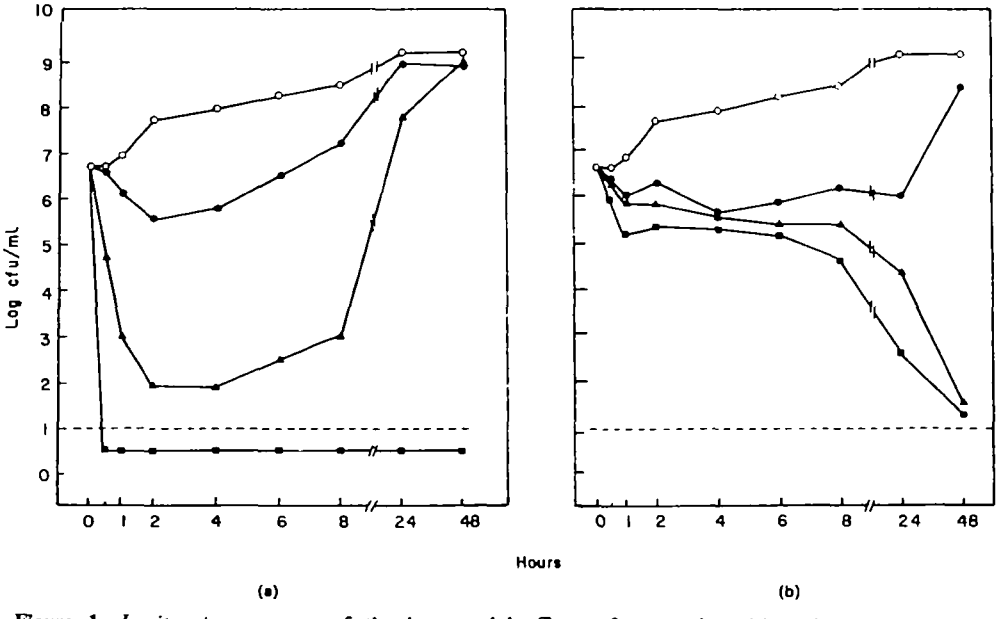

Figure 1. *In-vitro* time course of the bactericidal effects of gentamicin (a) and ticarcillin (b) on *Ps. aeruginosa* ATCC 27853 in calcium- and magnesium-supplemented MHB. Standard MICs (MBCs) for 6×10^5 organisms were 2 (8) and 32 (32) mg/l of gentamicin and ticarcillin, respectively ○, Control; ●, half-fold MIC; ▲, two-fold MIC, ■, ten-fold MIC.

several hours to exert a 99% bactericidal effect which was poorly concentration-dependent No break-through growth was observed at levels greater than the MICs of ticarcillin.

Comparative killing patterns in vivo

Killing patterns similar to those obtained *in vitro* were observed with high dose treatment of *Ps. aeruginosa* ATCC 27853 in the thigh infection model using granulocytopenic mice (Figure 2). Gentamicin resulted in >90% bacterial killing in the first 6 h of treatment, but a significant break-through growth was observed thereafter, although treatment continued and plasma levels far above the MIC were obtained, at least intermittently. The bactericidal effect of ticarcillin on the other hand was slow and bacterial break-through growth did not occur up to 24 h of high dose treatment.

Additional experiments were performed with lower doses of drug to see the effect of dosage schedule on antipseudomonal activity *in vivo* (Figures 3 and 4).

Importance of dosage schedules of gentamicin

Experiments with gentamicin (Figure 3) clearly revealed the key importance of peak levels for the antimicrobial effect of this drug. By 3 h of treatment, a single injection of 15 mg/kg of gentamicin (resulting in peak plasma levels of approximately 20 mg/l) was clearly more effective than the same total amount of drug subdivided in three fractional doses injected every hour and resulting in peak plasma levels of

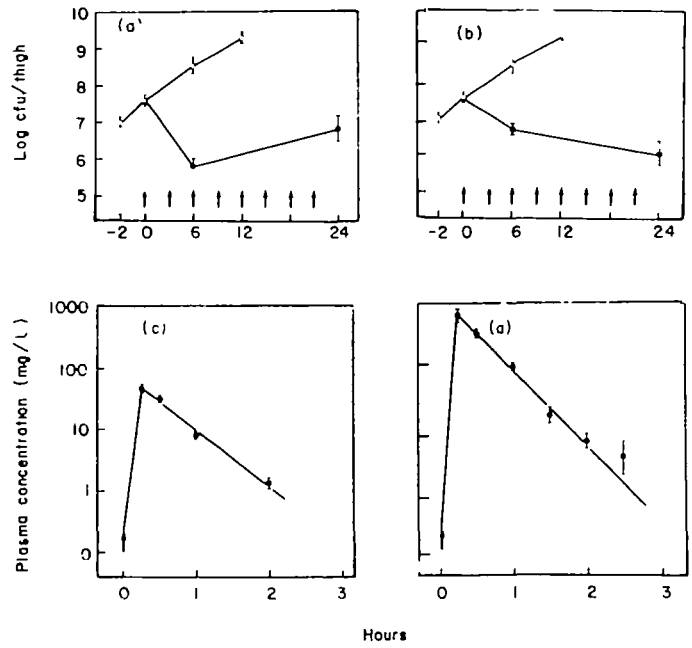

Figure 2. Comparative *in-vivo* killing patterns of a 3-h schedule of gentamicin (a), and ticarcillin (b), on *Ps aeruginosa* ATCC 27853 (granulocytopenic mice) The plasma kinetics of a single dose of gentamicin and ticarcillin are shown on a logarithmic scale in the (c) and (d) Each point represents the mean value ±S.D of three mice See Figure 1 for MIC and MBC-values

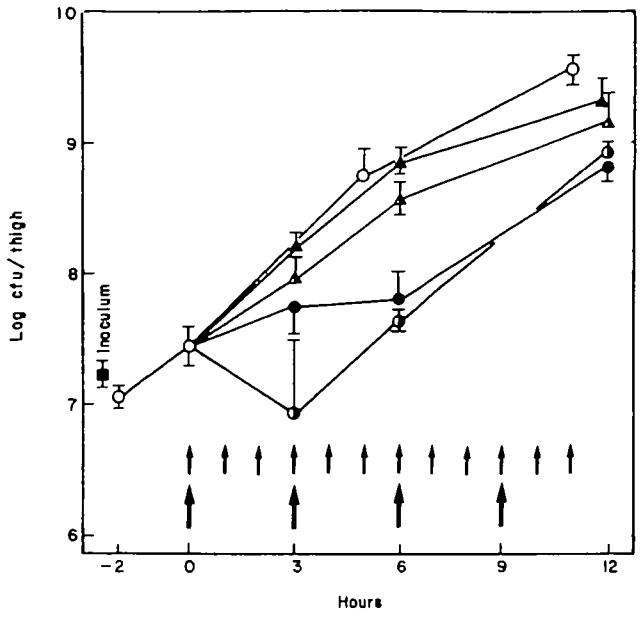

Figure 3. Importance of dosage schedules of gentamicin on *Ps aeruginosa* ATCC 27853 *in vivo* (granulocytopenic mice)

The effects of two 1-h regimens are compared to two 3-h regimens. Identical total amounts of drug had been injected in comparatively treated mice after 3, 6, 9, and 12 h. ○, Control; ▲, 1·67 mg/kg q 1 h; ◭, 5 mg/kg q 3 h, ●, 5 mg/kg q 1 h, ◐, 15 mg/kg q 3 h.

approximately 6–8 mg/l. Break-through growth could not be prevented by either of the two gentamicin dosage regimens.

Importance of dosage schedules of ticarcillin

The time course of the antipseudomonal effect of ticarcillin and the impact of dosage intervals of this drug were studied in granulocytopenic mice infected simultaneously with two clinical isolates of *Ps. aeruginosa* which differed substantially in virulence and susceptibility to ticarcillin (Figure 4). In contrast to strain E 29/2, strain 14974 was unable to grow in the thigh muscle of non-granulocytopenic mice. *In vitro* as well as in granulocytopenic mice strain 14974 multiplied slower than strain E 29/2. The colonies formed by the two strains on agar plates (small for strain 14974, larger and mucoid for strain E 29/2) could easily be distinguished.

Identical inocula of the two strains were injected into left and right thighs, respectively, of the same mice. Treatment was started 2 h later at a 1 h vs. a 3 h schedule, using identical total amounts of drug in both groups of mice treated. Against both *Ps. aeruginosa* strains tested the only effect of the 3 h regimen was to slow down bacterial growth. However, a complete bacteriostatic or bactericidal effect was not obtained, although peak plasma levels exceeded by at least two-fold the MIC for strain E 29/2 and by 64-fold the MIC for strain 14974. One hour injections of fractional doses of the same total amount of ticarcillin proved to be significantly more efficaceous against both strains tested despite three-fold lower peak plasma levels which, for strain E 29/2, did not even reach the MIC. Thus, the activity of ticarcillin was mainly

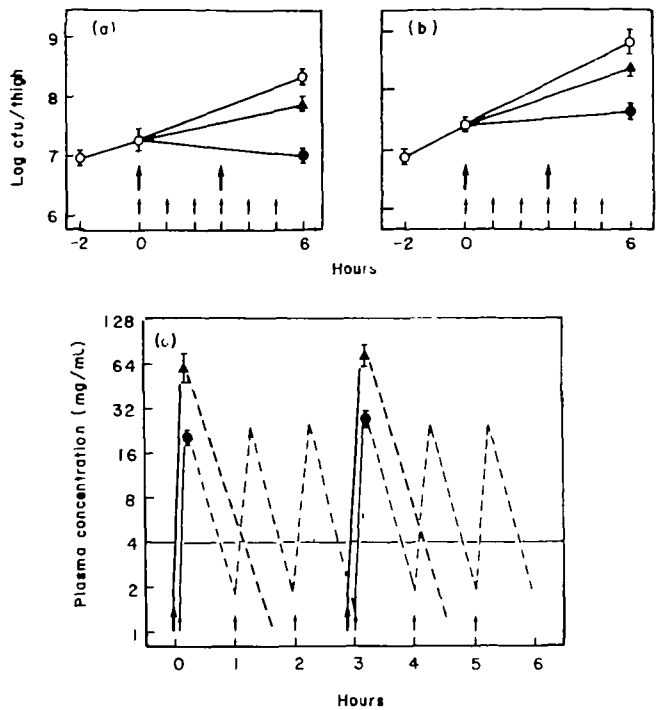

Figure 4. Importance of dosage schedules of ticarcillin on activity against two clinical isolates of *Ps aeruginosa* (a) 14974, MIC 1 mg/l; (b) E 29/2, MIC 32 mg/l Peak plasma levels of ticarcillin and presumptive kinetics of the two dosage regimens is shown in (c). ○, Control; ▲, 90 mg/kg q 3 h; ●, 30 mg/kg 1 h Four mg/l of ticarcillin was the lower limit of detectability in the biological assay used Identical total amounts of drug had been injected in both regimens by 3 and 6 h of treatment

dependent on constant presence of the drug rather than on peak levels far in excess of the MIC.

Discussion

Our experiments clearly demonstrate the difference between gentamicin and ticarcillin regarding the time course of their pharmacological response *in vitro* as well as *in vivo*. *In vivo* the antipseudomonal activity of gentamicin proved to be dependent on peak plasma levels. This finding reflected the dose-response curve observed with gentamicin *in vitro* which showed that increasing concentrations of gentamicin increased both absolute bacterial killing and the speed of killing. In addition, Bundtzen *et al.* (1981) have demonstrated a PAE of gentamicin on *Ps. aeruginosa* which lasted 1·6–2·6 h. This effect may thus cover, at least in part, the period of sub-MIC plasma levels between doses and prevent early bacterial regrowth during that phase of treatment. In conclusion, a gentamicin-type response, i.e. peak-dependent killing and a PAE, may well indicate an advantage *in vivo* of high dose, long interval treatment over a low dose, short interval regimen of any antimicrobial agent. This interpretation of the present data is in full agreement with our previous work (Gerber *et al.* 1983) and with the results of recent investigations by Powell, Thompson & Luthe (1983) who found once daily doses of aminoglycosides to be more effective than more frequent injections.

Interestingly, in those studies once daily dosing of aminoglycosides was also less toxic than the conventional 8 h schedule.

Our *in-vivo* results with ticarcillin were not surprising. Bacterial killing *in vitro* was slow and showed a poor correlation with concentration. High peak levels greatly exceeding the MIC seem to be of little value *in vivo*. On the other hand, the half-life of ticarcillin in mice was shorter than 20 min. It is therefore possible that the action time was just too short to exert any bactericidal effect. Since the half-life in man is considerably longer than in mice, a drug showing a response similar to that of ticarcillin might be underestimated when investigated in mice (Gerber *et al.*, 1983). However, it should be remembered that ticarcillin clearly lacks a PAE on *Ps. aeruginosa in vitro* (Bundtzen *et al.*, 1981). Therefore, it is logical to conclude that any antibiotic showing a response like that of ticarcillin does require super-MIC drug levels at all times in order to exert its maximal antimicrobial activity *in vivo*. This activity must therefore be associated with trough plasma levels.

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